**DAIRY WATERS**
**(Coliform Group)**

**[Unless otherwise stated all tolerances are ±5%]**

**1. Laboratory Requirements** _____

a. CP, items 33 & 34 _____

b. Sample volume sufficient to assure 100 mL for testing sufficient air space for mixing (about ¾ full), if completely filled do not accept _____

c. Transported and maintained at 0-4.4C (temperature control [TC] required) _____

d. If samples are not refrigerated, transit not to exceed 6 hours (TC not required) _____

e. Transit time does not exceed 30 hours _____

f. Samples examined within 30 hours of collection or within 2 hours of receipt (item 1d) _____

**APPARATUS**

**2. CP, see items 1 - 32 (as necessary)** _____

**3. Sample Containers** _____

a. Borosilicate glass, plastic bottles or bags _____

b. Sterile, containing 0.1 mL of 10% Sodium Thiosulfate _____

c. Holds sufficient sample with air space for all necessary bacterial tests _____

d. Maintains sample uncontaminated _____

**4. Incubator 35±0.5C (Make/Model _____)** _____

a. See CP item 15 for incubator requirements _____

**5. Fermentation Tubes/Bottles** _____

a. Sufficient size to conform with requirements for media, durham tube and sample _____

**6. Inoculation Equipment** _____

a. Sterilized loops of at least 3 mm diameter, 22-24 gauge nichrome, chromel or platinum-iridium wire _____

b. Disposable dry heat-sterilized hardwood applicator sticks, 0.2 to 0.3 cm in diameter and a minimum of 2.5 cm longer than the fermentation tubes _____

c. Inoculating needle _____

**7. Vacuum source with trap** _____

**8. Membrane filter funnel Brand** _____ _____

a. Free from defects that may interfere with function _____

b. Sterilizable _____

c. Marked at 100 mL, or pre-marked checked and adjusted, using a 100 mL Class A graduate cylinder _____

**9. Membrane cellulose filters, 47 mm, 0.45 µM (±0.02 µM), sterilized** _____

Brand _____ Lot # _____ _____

**10. Absorbent pads, sterilized Brand** _____ _____

**11. Forceps** _____

a. Round tipped, with smooth surface _____

**12. Culture (Petri) dishes (for MF) Brand** _____

Size _____ _____

a. Sterile with plastic, tight fitting covers _____

**13. Microscope and Lamp Brand** _____ **Model** _____ _____

a. Binocular, wide field, 10x oculars _____

b. Fluorescent light, adjacent, above, perpendicular to filter plane _____

c. Other optical device giving equivalent results _____

**CULTURE MEDIA**

**14. Storage of media** _____

a. See CP item 27 for media and storage requirements _____

b. MF Media

1. Store in dark at 0-4.4C _____

2. Broth medium used within 96 hr. Date prep. _____ _____

3. Plates kept no more than 1 week in a sealed container at 0-4.4C. Date prep. _____ _____

## TESTS FOR PRESENCE OF MEMBERS OF THE COLIFORM GROUP BY MULTIPLE-TUBE FERMENTATION TECHNIQUE

**15. Presumptive Test** _____

a. Lauryl Tryptose Broth _____

1. Before inoculating arrange tubes in order and label, or otherwise identify _____

2. Shake samples vigorously 25 times in a 30 cm arc in 7 sec before removing test portion _____

3. Remove test portions (100 mL total) within 3 min _____

4. Inoculate ten (10) fermentation tubes with 10 mL of sample or five (5) tubes with 20 mL with double strength LST or one bottle with 100 mL double strength LST _____

5. Incubate tubes at 35±0.5C for 24±2 hours _____

6. Examine tubes for gas - any gas is considered presumptive positive _____

7. Return negative tubes (no gas) to incubator and incubate an additional 24 hr (total of 48±3 hr) _____

8. Re-examine tubes for gas production after 48±3 hours _____

9. Record presence or absence of gas at each examination _____

10. Any gas produced by 24 or 48 hr is considered positive for the Presumptive Test _____

11. No gas after 48 hr is Not Found (NF) for the Test _____

12. Do not report gas production after 51 hr of incubation _____

13. Promptly submit all presumptive positive tubes showing gas production at 24 or 48 hr to the Confirmed Test _____

**16. Confirmed Test** _____

a. Brilliant Green Lactose Bile Broth

1. Gently shake presumptive positive tube _____

2. Transfer (loop or stick) portion of positive broth to BGLB broth _____

3. Incubate tubes at 35±0.5C for 24±2 hr _____

4. Examine tubes for gas - any gas is considered positive _____

5. Return negative tubes (no gas) to incubator and incubate an additional 24 hr (total of 48±3 hr) _____

6. Re-examine tubes for gas production after 48 hours _____

7. Record presence or absence of gas at each examination _____

8. Any gas produced by 24 or 48 hr is considered positive for the Confirmed Test _____

9. No gas after 48 hr is Not Found (NF) for the Test _____

10. Do not report gas production after 51 hr of incubation _____

**17. Reporting** _____

a. Report results of fermentation tubes that confirm as positive, reported as MPN/100 mL (Ó#1.1/100 mL if 10 mL in 10 tubes or 20 mL in 5 tubes are used) or Ó#1/100 mL if 100 mL presence/absence test used _____

b. If one or more tubes turbid with no gas production, invalidate the sample and request a re-sample from the same point source for heterotrophic plate count _____

c. Interpretation: for multiple tubes, Not Found (NF)is < 1.1/100 mL and Positive is Ó#1.1/100 mL; for presence/ absence, NF is < 1/100 mL and Positive is Ó 1/100 mL _____

**TESTS FOR PRESENCE OF MEMBERS OF THE COLIFORM GROUP BY MEMBRANE FILTRATION TECHNIQUE**

**18. Filtration** _____

a. Place (with alcohol flamed forceps, item 11) sterile membrane filter (item 9) on porous plate, secure funnel _____

b. Pour 100 mL test sample into funnel (item 8) and apply vacuum _____

c. After test volume has been filtered, rinse funnel by filtering 3 volumes of 20-30 mL of sterile buffered water _____

d. Turn off vacuum and remove filter with sterile (alcohol flamed) forceps _____

e. M-endo Broth _____

1. Sterile pad (item 10) placed in culture dish _____

2. Saturate pad with 2.0 mL of M-endo Medium, CP item 27n _____

3. Allow to stand a few minutes before pouring off excess _____

4. Prepared filter rolled (grid side up) onto pad slowly to avoid trapping air bubbles, do not drag across side of plate _____

f. M-endo Agar _____

1. Use culture dish previously prepared (CP item 27m) _____

2. Prepared filter placed on agar with rolling motion to avoid trapping air bubbles _____

**19. Incubation** _____

a. In saturated humidity, with dish inverted _____

b. At 35±0.5C for 21±1 hr _____

**20. Counting** _____

a. Count all sheen colonies as typical coliforms and dark suspect colonies as atypical coliforms, keep separate counts of each morphological type until confirmed _____

b. Confirm 10% up to a maximum of 10 isolated colonies, with representative proportions of each colony type _____

**21. Confirmation Test** _____

a. Make serial transfers of colonies to individual LST and then to BGLB tubes using the same transfer needle/stick _____

b. Incubate tubes at 35±0.5C for 24±2 hr _____

c. Examine tubes for gas _____

1. LST tubes with gas must be transferred to fresh BGLB tubes if the original BGLB tubes show no gas _____

d. Return negative tubes (no gas) to incubator and incubate an additional 24 hr (total of 48±3 hr) _____

e. Re-examine tubes for gas production after 48 hours _____

f. Record presence or absence of gas at each examination _____

g. Any gas produced in BGLB tubes by 24 or 48 hrs is considered positive for the Confirmation Test _____

h. No gas after 48 hr is Not Found (NF) for the Test _____

i. Do not report gas production after 51 hr of incubation _____

**22. Reporting** _____

a. Report confirmed colony count/100 mL _____

b. Invalidate all samples with confluent growth or TNTC, and request a re-sample from the same point source for heterotrophic plate count _____

c. Interpretation: Not Found (NF) is < 1/100 mL and Positive is Ó#1/100 mL _____

**HETEROTROPHIC BACTERIA**
**STANDARD PLATE COUNT METHOD**

**23. Heterotrophic Plate Count Method** _____

a. Plate samples as in SPC, items 2-10, 13 and 14 _____

b. Incubate at 35±0.5C for 48±3 hours _____

c. Count as in SPC item 16-17 _____

d. Report counts as in SPC item 20 _____

e. Record as "Heterotrophic Plate Count/mL at 35C" _____

f. Interpretation: Negative if < 500 CFU/mL and Positive if Ó#500 CFU/mL _____

**CHROMOGENIC SUBSTRATE (MMO-MUG) PRESENCE - ABSENCE SCREENING TEST FOR DAIRY WATERS (SOURCE WATER SUPPLIES <u>ONLY</u>)**

**24. Materials** _____

a. Color comparator _____

b. Sterile borosilicate glass or clear plastic bottles to contain 100 mL sample with sufficient air space for mixing (about ¾ full) _____

c. MMO-MUG substrate, see CP item 27o _____

d. Quality control procedures conducted on each lot of substrate received, as recommended by manufacturer, test by spiking with known coliform, records maintained _____

**25. Procedure** _____

a. Aseptically add pre-weighed MMO-MUG substrate to 100 mL of water sample _____

b. Optionally, add 100 mL sample to the MMO-MUG substrate in a sterile container provided by the manufacturer _____

c. Aseptically cap and mix thoroughly by inverting 25 times to dissolve reagent (does not completely dissolve) _____

d. Incubate at 35±0.5C for a **minimum** of 24 hours, not to exceed 28 hours _____

e. Examine containers for the production of yellow color _____

**26. Interpretation** _____

a. If no yellow color is observed _____

1. Record sample as Not Found (NF) for total coliforms _____

2. Report as total coliform Not Found (NF) in 100 mL sample: < 1/100 mL _____

b. If yellow color present _____

1. Gently invert container several times until color is uniformly dispersed through the sample _____

2. Compare yellow color to color comparator dispersed into the **SAME** type of sample container _____

3. If color is equal to or greater than that of the color comparator, sample reported as Positive for total coliforms _____

4. If color is obvious but less than the comparator, sample reported as Not Found (NF) _____

5. Report as total coliforms present in 100 mL sample: Ó#1/100 mL _____

**CHROMOGENIC SUBSTRATE (MMO-MUG) MULTIPLE TUBE PROCEDURE FOR THE PRESENCE OF TOTAL COLIFORMS (SOURCE WATER SUPPLIES ONLY)**

**27. Materials, see items 24 a-d)** _____

**28. Procedure** _____

a. Before transferring sample portions arrange tubes in order and identify _____

b. Shake samples vigorously 25 times in a 30 cm arc in 7 sec _____

c. Aseptically add pre-weighed MMO-MUG substrate to 100 mL sample _____

d. Optionally, add 100 mL of sample to container with MMO-MUG substrate provided by manufacturer _____

e. Aseptically cap and mix thoroughly by inverting 25 times to dissolve reagent (does not completely dissolve) _____

f. Remove test portions (100 mL total) within 3 minutes _____

g. Transfer 20 mL of sample/reagent mixture to five tubes, or 10 mL to ten tubes _____

h. Optionally, transfer 100 mL of mixed (see item 28b) sample to 10 tubes containing pre-dispensed MMO-MUG reagent provided by manufacturer _____

i. Incubate tubes at 35±0.5C for a **minimum** of 24 hours, do not to exceed 28 hours _____

j. Examine tubes for the development of yellow color _____

1. Mix tubes to uniformly distribute yellow color _____

2. Compare tubes to color comparator tube (**SAME** size and type as MPN tubes) _____

3. Tubes with color equal to or greater than color comparator tube recorded as Positive _____

4. Tubes with obvious color but less than comparator, sample reported as Not Found (NF) _____

**29. Reporting** _____

a. If all tubes show no color, report as Not Found (NF): < 1.1/100 mL _____

b. If one or more tubes show yellow color (see 28j) report as Positive: Ó 1.1/100 mL _____

**CHROMOGENIC SUBSTRATE PRESENCE (XGAL - MUG) - ABSENCE SCREENING TEST FOR DAIRY WATERS (SOURCE WATER SUPPLIES ONLY)**

**30. Materials** _____

a. E*Colite substrate, see CP item 27p _____

b. Quality control procedures conducted on each lot of substrate received, as recommended by manufacturer, test by spiking with known coliform, records maintained _____

**31. Procedure** _____

a. Add water sample to the E*Colite substrate _____

1. Tear perforated strip _____

2. Open bag by pulling white tabs _____

3. Aseptically pour 100 mL of water sample into bag (do not touch inside of bag) _____

4. Flatten bag to remove air _____

5. Twirl bag 2-3 times around twister wires to form a leak proof seal _____

6. Fold twisters around back of bag _____

7. Shake bag 25 times in 7 seconds to dissolve sodium thiosulfate tablet, if present _____

8. Continue rolling to build pressure in water compartment _____

9. Maintain pressure on rolled area and push water through first seal into powder section of bag **ONLY** _____

10. Shake bag 25 times in 7 seconds to completely dissolved powder in water (push mixture against bag sides to pull apart any remaining seal) _____

b. Place sealed bag in 35C water bath for 10 minutes _____

c. Transfer to 35±0.5C incubator for 28 hours _____

d. Examine bags for the production of blue or blue/green color or blue color in corners of bag _____

**32. Interpretation** _____

a. If yellow color is observed: _____

1. Record sample as Not Found (NF) for total coliforms _____

2. Report as total coliform Not Found (NF) in 100 mL sample: < 1/100 mL _____

b. If blue or blue/green (or blue in corners) color observed: _____

1. The sample is Positive for total coliforms _____

2. Report as total coliforms present in 100 mL sample: Ó#1/100 mL _____

**MISCELLANEOUS**

**33. Copy of current in-use edition of <u>Standard Methods for the Examination of Water and Wastewater</u> in laboratory** _____